REMARKS

Claims 27 through 45 were presented for examination in the present application. The instant amendment cancels claims 28 and 38 without prejudice. Thus, claims 27, 29 through 37, and 39 through 45 are presented for consideration upon entry of the instant amendment.

As noted above, claims 28 and 38 have been cancelled. Reconsideration and withdrawal of the rejections to claims 28 and 38 are respectfully requested.

Claims 27 through 45 were rejected under 35 U.S.C. 112, second paragraph.

Claim 27 has been amended accordingly so as to overcome the rejection. Claims 29 through 36 depend from independent claim 27. As such, the rejections to claims 29 through 36 are also overcome. Reconsideration and withdrawal of the rejections to claims 27 and 29 through 36 are respectfully requested.

Claim 37 has been amended accordingly so as to overcome the rejection. Claims 39 through 45 depend from independent claim 37. As such, the rejections to claims 37 and 39 through 45 are also overcome. Reconsideration and withdrawal of the rejections to claims 37 and 39 through 45 are respectfully requested.

Claims 37 through 45 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement.

The Office Action asserts that "in view of the experimental method and results, there is no support of using the above mentioned ACE isoform biomarkers as indicators of renal damage". See, page 4. Applicants respectfully assert that it was known/inherent at the time of the invention that when analyzing human urine in healthy subjects with family history of hypertension, for the presence of the 190kDa, 90kDa, and 65kDa ACE isoforms, the alteration in expression levels of one of these proteins could indicate renal failure. The aim of the studies was to evaluate the expression of ACE isoforms in urine of patients with diabetes mellitus.

In order to aid in showing that an alteration in the expression of ACE isoforms in urine was also indicative of renal lesions, Applicants submit below a population study of ACE isoforms in the organ damage during diabetes evolution.

Applicants studied five groups of diabetic patients from the Division of Diabetes from the Endocrinology Department of UNIFESP. This protocol was approved by the Ethics Committee of UNIFESP. The Groups were as follows:

Group I (N-N): Patients with Diabetes type I, normotensives without microalbuminure (n=45);

Group II (M-N): Patients with Diabetes type I, normotensives with microalbuminure (n=35);

Group III (N-H): Patients with Diabetes type I, hypertensives without microalbuminure (n=29);

Group IV (M-H): Patients with Diabetes type I, hypertensives with microalbuminure (n=31); and

Group V (MP): Patients with Diabetes type I, hypertensives with macroproteinure (n=28).

The patients were given a questionnaire asking for the family history of hypertension and diabetes. After evaluation of blood pressure (MAPA), blood and urinary samples were collected in the morning for future assays. The blood samples were used for glycemia, urea, creatinine, hemoglobin glycosilate, lipid profile and angiotensins. Urinary samples were used for microalbuminure/albuminure, creatinine, ACE activity determinations and expression.

Applicants analyzed the demographic, antropometric and biochemistry characteristics of the studied population. In the pressoric data and in biochemistry profile, Applicants detected differences between the groups. The results are in Table 1 (below).

Table 1. Demographic and biochemistry profiles.

CHARACTERISTICS	N-N N=45	M-N N=35	N-H N=29	M-H N=31	MP N=28
Age (years)	17,8 ± 0,52	18,6 ± 0,60	21,9 ± 1,20 #	23,4 ± 1,30	30,6 ± 1,50 &§
Diabetes Time(months)	76. 8 ± 8,4	101.1 ± 8.5	142,3 ± 15.4 #	154,5 ± 14,5	235,3 ± 13,4&§
Urinary Creatinine	0,79 ± 0,02	0,88 ± 0,03	0,98 ± 0,03	0,98 ± 0,04	1,56 ± 0,13 &§
Creatinine Clearence	113,1 ± 4,7	111,3 ± 4,1	113,3 ± 4,6	110,3 ± 7,2	64,9 ± 7,0 &§
Microalbuminuria	5,46 ± 0,73	46,5 ± 6,1	8,9 ± 1,1	80,7 ± 19,3	801,9 ± 175,4 &§
SBP (mmHg)	110,9 ± 0,9	114,9 ± 0,9 *	130,3 ± 1,2 #	133,5 ± 1,2	137,5 ± 2,0 §
DBP (mmHg)	70,6 ± 0,8	74,9± 1,0 *	84,8 ± 0,9 #	86,5 ± 1,0	89,6 ± 1,1§

The results are expressed as mean ± SEM. SBP – Sistolic blood pressure DBP – Diastolic blo pressure;. Variance test (ANOVA) followed by the Tukey-Kramer. * P<0,05 N-N compared with I N; # P<0,05 N-N compared with N-H e &P<0,05 M-H compared with MP; § P<0,05 MP compar with N-N, M-N, N-H.

In Table 2 (below), the total urinary ACE activity (total activity equals sum of somatic plus N-domain activities) from patients of group MP was increased significantly for both studied substrates. Also, AII (angiotensin II, product of ACE isoforms) levels were increased in this group. The activity was corrected by creatinine excretion.

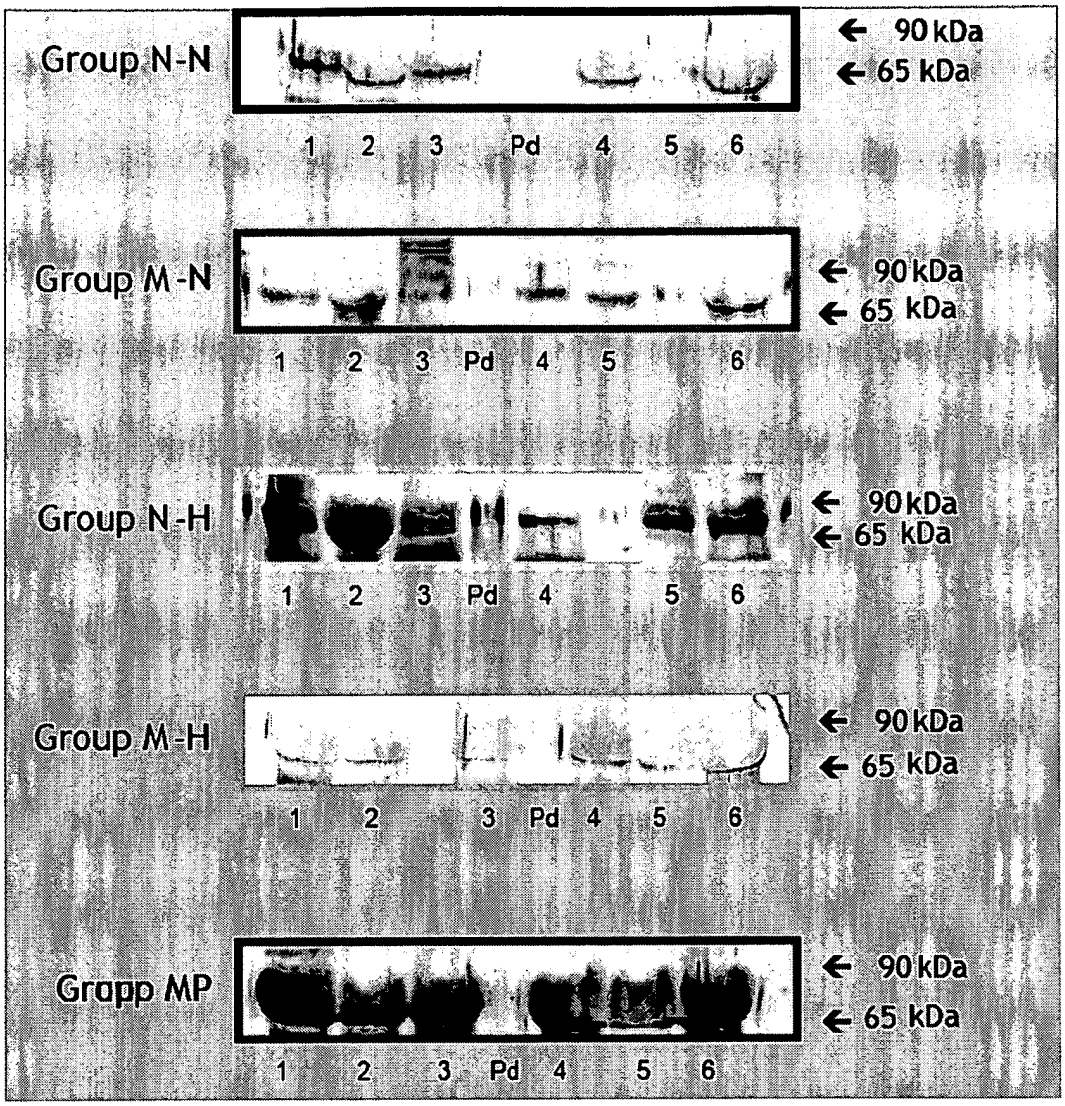
Table 2. Urinary activity of ACE and circulant levels of angiotensin II.

	N-N N=45	M-N N=35	N-H N=29	M-H N=31	MP N=28
ACE activity ZPH/creat	3,7 ± 0,7	5,8 ± 1,8	9,6 ± 3,2	10,1 ± 4,3	45,5 ± 20,3 &§
ACE activity HHL/creat	2,9 ± 0,6	4,2 ± 1,4	7,2 ± 2,6	7,1 ± 3,0	29,3 ± 14,2 &
Angiotensin II	0,42 ± 0,04	2,30 ± 0,23 *	2,11 ± 0,35 #	0,95 ± 0,20	3,31 ± 0,40 &

Results express as mean SEM. Test of Variance (ANOVA) followed by the Tukey-Kramer test, * $P<0,05$ N-N compared with M-N; # $P<0,05$ N-N compared with N-H e &$P<0,05$ M-H compared with MP; § $P<0,05$ MP compared with N-N, M-N, N-H.

The increase of ACE activity is correlated with the results found in analysis utilizing Western Blotting of ACE isoforms. In Figure, 1 the Applicant observed an increase of 90 kDa and also of 65 kDa ACE.

Figure 1 - *Western blotting* using anti-ACE policlonal antibody. We analyzed samples from 5 patients in each group (1 a 5); Pd: standard molecular mass.



Urinary ACE activity has a tendency to increase during microalbuminure evolution, being elevated in macroproteinuric patients (Table 2). Analysis of ACE isoforms expression indicated a positive correlation between N-domain proteic expression analyzed by *Western Blotting*, and the total activity, determined using ZPheHL as substrate. The inventors suggest renal damage in these patients based on alterations in activity and expression of ACE isoforms levels.

Patients with MA present significant abnormalities in the kidney, including glomerulus. Studies correlating lesions in renal structure and presence of MA indicated a relationship between renal structure and albuminuria (Osterby, Hartmann *et al.*, 2002). The renal lesions advance over the years of DM and are localized in different portions of the kidney, as for example glomerulus, tubules, etc.

Many new markers have been proposed as risk factors of renal damage, despite the use of microalbuminure as a control (Poulsen, 2002). The increase in urinary ACE activity and expression can predict hypertension, and also renal damage as visualized in Table 2 and Figure 1. The Western Blotting can be used in future, e.g., to monitor ACE isoforms proteins expression in tissues after byopsia.

As such, Applicants respectfully submit that the enablement requirement of the method recited by claim 37 is met in the present application. Claim 37 is in condition for allowance. Claims 39 through 45 depend from independent claim 37, and are in condition for allowance for at least the reasons given above for claim 37. Reconsideration and withdrawal of the rejections to claims 37 and 39 through 45 are respectfully requested.

Claims 27 through 31 and 34 were rejected under 35 U.S.C. 103(a) as being unpatentable over Casarini et al. (Intl. J. Biochem Cell Biology 2001 Vol. 33, page 75-85) ("Casarini"). Claims 32, 33, 35, and 36 were rejected under 35 U.S.C. 103(a) as being unpatentable over Casarini in view of U.S. Patent Publication No. 20050147600 ("Acton").

Claim 27 now recites "A method of detecting a predisposition for the development of hypertension in an individual, comprising detecting a presence of the following three angiotensin converting enzyme isoforms in an aliquot of fresh or concentrated biological fluids, cells or tissues obtained from the individual, wherein the three angiotensin converting enzyme isoforms are 65kDa, 90 kDa, and 190kDa, and wherein the 65kDa and 190kDa isoforms are present in a normotensive patient, and wherein the 65kDa, 90kDa, and 190kDa isoforms must be present to indicate the predisposition for the development of hypertension (emphasis added)".

Casarini discloses the purification and characterization of ACEs from human urine of hypertensive patients. This document describes the purification of urine by DEAE-cellulose cellex D followed by gel filtration. The purified enzymes were then characterized by polyacrylamide gel electrophoresis and also by Western Blotting. Casarini discloses that the enzymes with 90 and 65 kDa presented in urine of hypertensive patients differs from the urine of normotensive subjects which presents the 190 and 65 kDa forms. Based on Casarini, it is not possible to predict the identification of the three enzymes at the same time.

In addition, Casarini does not describe in detail the method of urine preparation. The claimed present application has differences in the concentration and dialysis steps of urine preparation, which is not described in Casarini. As such, Applicants respectfully submit that it be impossible for a person having ordinary skill in the art to utilize the disclosure of Casarini so as to predict that the the presence of

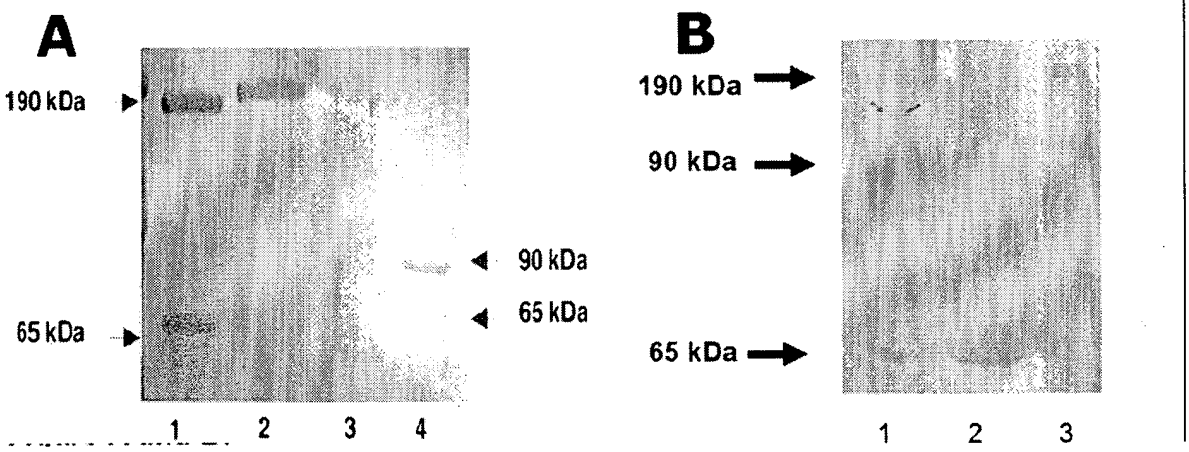
the three isoforms together could give a profile to predict hypertension and, also that the 90 kDa isoform could be used as a marker of hypertension.

Only by having information of the family history of hypertension and by detecting the presence of the three enzymes at the same time utilizing the diagnostic assays disclosed in the present invention, would it be possible to diagnose or confirm that a symptomatic subject (e.g. a subject symptomatic for hypertension), has a genetic defect (e.g. in an ACE gene or in a gene that regulates the expression of an ACE gene), which causes or contributes to the particular disease or disorder, such as hypertension.

Alternatively, the information can be used prognostically for predicting whether a non-symptomatic subject is likely to develop a disease, which is caused by or contributed to an abnormal ACE activity or protein level (e.g. hypertension). In addition, it is important to note that knowledge of the particular alteration or alterations, resulting in the presence of the 190, 90 and 65 kDa ACE proteins in an individual, alone or together with information on other genetic defects contributing to the same disease (the genetic profile of the particular disease) allows customization of therapy for a particular disease to the individual's genetic profile.

Therefore, Applicants respectfully submit that based solely on the disclosure of Casarini, it would not be possible to obtain the profile described in the claimed present invention.

Applicants submit below Figure A from Casarini, and Figure B that represents the test results utilizing the claimed method of the present application. Although Figure B was not included in the present application, Applicants respectfully submit that no new matter has been added as this Figure merely shows a test result of the presently claimed method. Should the Examiner require, Applicants would agree to submit Figure B in a Declaration. In addition, Applicants would like to point out that Casarini does not point to the presence or absence of family history of hypertension that was described in the present invention.



A, Western Blot of fresh urine from healthy and hypertensive subjects. SDS polyacrylamide gel electrophoresis (7.5% acrylamide) followed by Western blotting analysis with polyclonal antiserum (Y4) raised against human kidney ACE.Lane 1, ACE isoforms in fresh urine from normal subjects; lane 2, wild-type recombinant ACE; lane 3, wild-type secret recombinant ACE; lane 4, ACE isoforms in fresh urine from mild hypertensive patients **(example from Casarini)**.

B, Western Blot of fresh urine from healthy subjects with family history of hypertension. SDS polyacrylamide gel electrophoresis (7.5% acrylamide) followed by Western blotting analysis with monoclonal antibody 9B9 specific for N-domain portion of ACE raised against ACE.Lane 1, ACE isoforms in fresh urine from normal subject 1 with familiar history of hypertension; lane 2, ACE isoforms in fresh urine from normal subject 2 with familiar history of hypertension; lane 3, protein standards. The arrows indicate the positions of the protein molecular mass (in kDa).

Casarini discloses "These results suggest that this recently found form of ACE (90 kDa), present only in the urine of hypertensive patients, could have an important physiologic role in hypertension". See, page 84. Applicants respectfully submit that from this statement alone, it would be impossible for one having ordinary skill in the art to propose a method of detecting a predisposition for the development of hypertension based on identifying the presence of the three ACE isoforms.

Casarini disclosed the purification and characterization of ACEs from human urine of hypertensive patients. Specifically, Casarini discloses the purification of urine by DEAE-cellulose cellex D followed by gel filtration. The purified enzymes were thereafter characterized by polyacrylamide gel electrophoresis and also by Western Blotting. Casarini discloses that the presence of enzymes with 90 and 65kDa in the urine of hypertensive patients differs from the urine of normotensive subjects having isoforms of 190 and 65 kDa. Based on Casarini, it is not possible to predict the identification of the three enzymes at the same time.

Also, Casarini did not describe in detail the method of urine preparation. The present application has differences in the concentration and dialysis steps of urine preparation, which are not described in Casarini. Therefore, it is impossible for a person having ordinary skill in the art to assess the use of the information with the same purpose on the present application, namely, that these isoforms together could give a profile to predict hypertension and also that specially the isoform of 90 kDa coud be used as a marker of hypertension.

It is only by having information about the family history of hypertension and the detection of the three enzymes at the same time using the diagnostic assays described herein, it is useful for diagnosing or confirming that a symptomatic subject (e.g. a subject symptomatic for hypertension), has a genetic defect (e.g. in an ACE gene or in a gene that regulates the expression of an ACE gene), which causes or contributes to the particular disease or disorder, such as hypertension.

As such, Applicants respectfully submit that one having ordinary skill in the art at the time of the invention, would not find the method of presently pending claim 27 obvious based on Casarini. Claim 27 is in condition for allowance. Claims 29 through 36 depend from independent claim 27 and are in condition for allowance for at least the reasons given above for claim 27. Reconsideration and withdrawal of the rejections to claims 27 and 29 through 36 are respectfully requested.

In view of the above, it is respectfully submitted that the present application is in condition for allowance. Such action is solicited.

If for any reason the Examiner feels that consultation with Applicants' attorney would be helpful in the advancement of the prosecution, the Examiner is invited to call the telephone number below.

Respectfully submitted,

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